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Pesticide Rebuttable Presumption against Registration. Piperonyl Butoxide Decision Document

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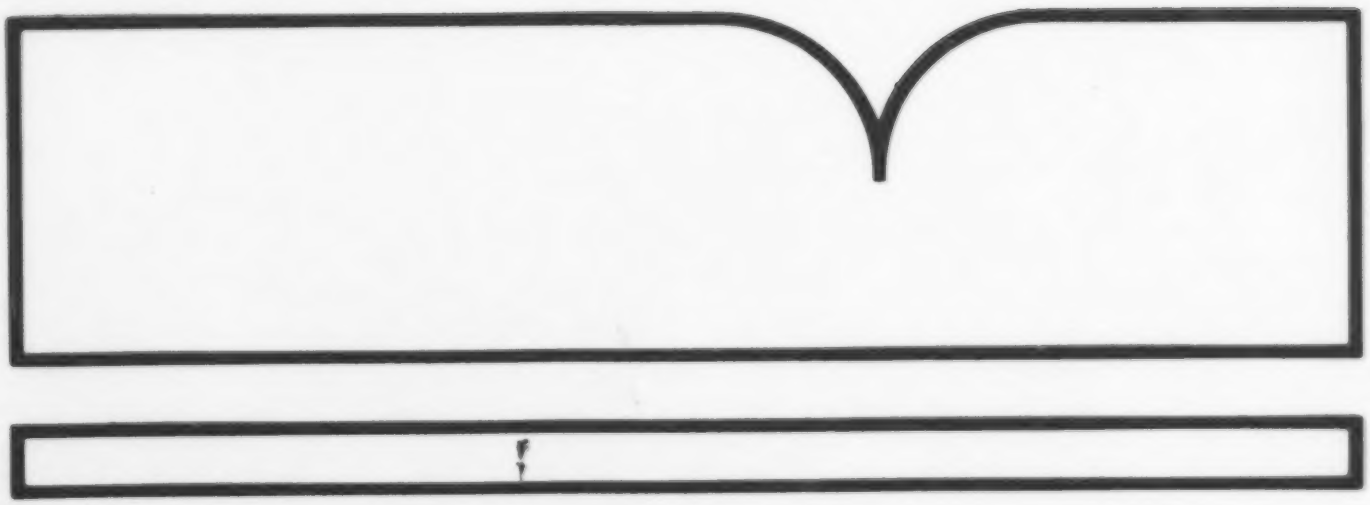

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PIPERONYL BUTOXIDE

DECISION DOCUMENT

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Executive Summary

Piperonyl butoxide is an insecticide synergist principally employed to enhance the activity of natural pyrethrum. It is contained in approximately 4,200 federally registered formulated products manufactered by over 900 registrants. Although in recent years there has been a trend toward the use of unsynergised synthetic pyrethroid formulations, piperonyl butoxide, in combination with pyrethrum, remains common in the marketplace. Piperonyl butoxide is formulated into hand held aerosols, pressurized sprays, total release pressurized products, intermittent aerosols, fogging concentrates, emulsions, dusts, and a number of additional specialty products.

Piperonyl butoxide first came to the attention of the Agency because of its appearance on the Mrak list (1969) of chemicals. This list indicated chemicals warranting additional study with regard to carcinogenicity. The Mrak Commission based their assessment upon a study by Innes et al. (1969), which reported tumors in one of the two mouse strains tested. Piperonyl butoxide was, thus, referred to the Agency for scientific review in July of 1976. It was identified as a compound requiring intensive scientific review in July 1978 (43 FR 30613).

Subsequent to the initiation of the Agency's review, the National Cancer Institute (NCI) tested piperonyl butoxide for carcinogenicity in both rats and mice. The NCI report (1979) found increases in the incidence of lymphomas in female rats and the incidence of lacrimal gland adenomas in male mice. Although of statistical significance when viewed from the perspective that the incidence of lymphomas in the control rats was low by chance in comparison to the historical spontaneous incidence of lymphomas in the rat strain tested, the NCI suggested that had the experimental group been compared to the historical controls there would have been no significant difference. NCI did not, however, perform a statistical test to confirm their hypothesis.

With regard to the mouse study, the NCI stated that "...adenomas of the eye or lacrimal gland occurred at incidences that were dose related, but in direct comparison the incidences in the individual dosed groups were not significantly higher than that in the control group..., thus, the occurrence of this tumor in male mice was not clearly related to the administration of the test chemical." NCI later indicated that, historically, spontaneous adenomas of the eye or lacrimal gland normally occur at a higher frequency than in the matched controls in this study, and that, therefore, one could not call piperonyl butoxide a carcinogen based on the dose related incidences. NCI concluded that under the conditions of their bioassay, piperonyl butoxide was not carcinogenic in the strains of rats and mice tested.

The NCI study was additionally reviewed by the Hazard Evaluation Division (HED), of the Office of Pesticide Programs (OPP/EPA). The HED review found a statistically significant increase in the occurrence of lymphomas in female rats when compared to matched controls and a significant increase in the occurrence of adenomas of the eye or lacrimal gland in male mice when compared to matched, pooled or historical controls.

With the conflicting assessment of piperonyl butoxide's carcinogenic potential, HED referred the data to EPA's Carcinogen Assessment Group (CAG). HED additionally referred four studies on possible mutagenic effects to EPA's Reproductive Effects Assessment Group (REAG).

CAG, upon review of the data, concluded that the Innes study provides one positive result for reticulum cell sarcoma in one strain of treated male mice through one route of administration. Regarding the NCI study, CAG disagreed with the NCI. CAG stated that the lymphoma incidence in the female rats, when evaluated by careful statistical analysis was significantly higher than that of matched controls, pooled controls, historical controls and controls cited by another auther (Goodman, 1979). The CAG further provided, however, that the male control rats in the NCI study had a very high incidence of lymphomas (45%). This, they said, casts suspicion upon the whole study and, thus, no conclusions could be reached concerning piperonyl butoxide's carcinogenicity.

Regarding the lacrimal gland adenomas found in the male mice, CAG stated that the numbers of tumors were small in the NCI study, and that the findings probably occurred by chance variation.

As a result of the multiple reviews, both CAG and HED have concluded that the available evidence is not sufficient to make a definitive judgement vis-a-vis piperonyl butoxide's potential for carcinogenicity. Both CAG and HED have agreed that further testing is necessary.

A related issue, cocarcinogenicity, was additionally identified as a potential cause for concern. Mrak (1969) reported that PB appeared capable of enhancing the toxic effects of certain substances and that one study (Epstein, 1967) suggested that it may be a cocarcinogen with Freon 112 and 113. Agency review of the Epstein study found no conclusive evidence of cocarcinogenicity.

With regard to mutagenicity, REAG could not make a definitive statement regarding PB because of inadequacies in all four studies reviewed. HED, as well as REAG, has concluded that additional testing must be undertaken.

Other data gaps have been identified by CAG and HED. These data gaps include product chemistry, mammalian metabolism, and reproductive effects. Both the product chemistry and metabolism data requirements are related, in part, to an understanding of piperonyl butoxide's potential for oncogenicity. The product chemistry data requirements are intended to identify synthesis processes and to establish the presence or absence of potentially oncogenic manufacturing impurities. The metabolism studies will be designed to facilitate interpretation of those data derived from feeding studies vis-a-vis the principal route of exposure, ie. inhalation. Reproductive effects data are being sought due to both the high exposure potential of individuals of reproductive age, and the current absence of valid reproductive effect data.

As Agency review of all available scientific literature has failed to conclusively establish that piperonyl butoxide either meets or exceeds established risk criteria, the Agency is returning piperonyl butoxide to the registration process. A Notice will be sent to all registrants informing them of the requirement to perform additional testing. These tests, the appropriate protocols and time schedules will be described in detail within the Notice.

I. Introduction

Section 3(a) of the Federal Insecticide Fungicide and Rodenticide Act [FIFRA] requires all pesticide products to be registered by the Administrator of EPA before they may be sold or distributed. Section 6(b) of FIFRA authorizes the Administrator to issue a notice of intent to cancel the registration of a pesticide or to change its classification if it appears that the pesticide or its labeling "does not comply with the provisions of [FIFRA] or, when used in accordance with widespread and commonly recognized practice, generally causes unreasonable adverse effects on the environment." Thus the Administrtator may cancel the registration of a pesticide whenever he or she determines that it no longer satisfies the statutory standard for registration, which requires, among other things, that the pesticide not cause "unreasonable adverse effects on the environment" [Section 3(c)(5) of FIFRA]. These "unreasonable adverse effects" are defined in Section 2(bb) of FIFRA to include "any unreasonable adverse effects to man or the environment, taking into account the economic, social and environmental costs and benefits of the use of any pesticide."

The Environmental Protection Agency, hereafter referred to as the Agency, created the Rebuttable Presumption Against Registration [RPAR] process to facilitate the identification of pesticide uses which may not satisfy the statutory standard for registration and to provide a public, informal procedure for the gathering and evaluation of information about the risks and benefits of these uses. The regulations governing the RPAR process are set forth in 40 CFR 162.11. In broad summary, these regulations set forth certain criteria of risk and provide that an RPAR shall arise against a pesticide if the Agency determines that the ingredient(s), metabolite(s), or degradation product(s) of the pesticide in question meet or exceed any of these risk criteria.

In administering the RPAR process, the Agency adheres to the standard for initiating the RPAR process established by Section 3(c)(8), one of the 1978 Amendments to FIFRA, which provides that the Agency may not start an RPAR unless it has "a validated test or other significant evidence raising prudent concerns of unreasonable adverse risk to man or the environment."

When the Agency publishes a notice indicating that an RPAR has arisen, the 40 CFR 162.11 regulations require that an opportunity then be provided for registrants, applicants, and interested persons to submit evidence to rebut the presumption, or evidence relating to the economic, social, and environmental benefits for any use of the pesticide. If the presumptions of risk are not rebutted, the evidence on the benefits of the pesticide is evaluated and considered along with the information on the risks. The Agency then analyzes various methods of reducing the amount of risk from the pesticide together with their costs and determines whether the pesticide can be regulated so that the benefits of continued use outweigh the risks. If measures short of cancellation cannot reduce the risks associated with any given use of the pesticide to a level which is outweighed by benefits, the use in question must be cancelled.

With regard to piperonyl butoxide [PB], two published documents, Innes et al. (1969) and Mrak (1969), revealed a potential oncogenic hazard. Innes et al. using a preliminary tumorigenic screening test, classified PB as a compound requiring additional study. Dr. Mrak, Chairman of an advisory committee to the Secretary of Health, Education, and Welfare (the Mrak Commission), supported this conclusion in his report on pesticides. Piperonyl butoxide was, thus, suspected by the U.S. Environmental Protection Agency of meeting 40 CFR 162.11 risk criteria. Although potential oncogenicity was the primary basis of concern, the Agency initiated a review of all available toxicological data. The potential effects examined in this decision document are oncogenicity, co-oncogenicity (co-carcinogenicity), mutagenicity, reproduction and teratogenicity. An additional concern involves the metabolic fate of PB in mammalian systems. This latter concern is not directly involved with any 40 CFR 162.11 risk criteria, but rather with the availability of those data necessary for an interpretation of chronic effects data.

This document presents the review of scientific data gathered to determine whether PB met or exceeded any of the risk criteria set out in 40 CFR 162.11. The Agency found no valid evidence to indicate that PB met or exceeded any of the risk criteria; however, there was not sufficient information to determine whether PB caused any reproductive, or mutagenic adverse effects. Results of studies addressing these toxic effects did not indicate immediate cause for concern. They did not, however, establish, with a reasonable degree of certainty, that PB is either nonmutagenic or nonteratogenic.

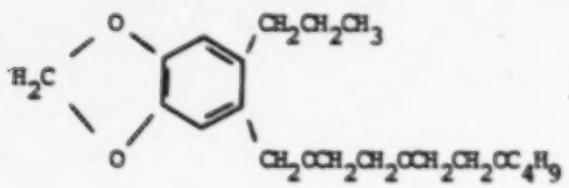
Those studies assessing carcinogenic effects were found to be inadequate. The Agency, therefore, can not reach a definitive conclusion concerning PB's potential as a carcinogen. Data gaps also exist in the areas of metabolism and product chemistry. PB has not, however, been found to meet or exceed any risk criteria. The Agency, therefore, will not initiate the RPAR process. Rather, the Agency is recommending that PB be returned to the registration process with the stipulation that the registrants conduct appropriate tests to provide data on carcinogenicity, mutagenicity, reproduction, metabolism, and product chemistry.

This decision document is divided into five sections. Section I is this introduction. Section II discusses general information on the product's chemistry, uses, and tolerances. Section III addresses the primary purpose of the review; it compares data on potential adverse effects of PB with the Agency's criteria for a Rebuttable Presumption Against Registration. Section IV summarizes the conclusions of this review of piperonyl butoxide and recommends actions to be taken as a result of these conclusions. Sections V and VI contain tables relating information on the cocacinogenicity of PB and Freons and the statistical significance of the Epstein et al. (1079a). Section VII is a bibliographical listing of the works cited.

II. Chemical Profile

A. Chemical Identity

The chemical name for piperonyl butoxide (PB) is (butyl carbitol) (6-propylpiperonyl ether). The Chemical Abstracts Service has assibned to PB the registry number 51-03-6. The structural formula of PB is shown below:



PB is a derivative of methylene-dioxyphenol (MDP). Compounds containing the MDP chemical group are widely distributed in essential oils, alkaloids, and other physiologically active compounds of natural and synthetic origin. Members of this group, PB among them, are used as synergists for pyrethrum and certain synthetic pyrethroid insecticides.

The manufacture of piperonyl butoxide comprises three chemical reactions (Brown et al., 1970):

- -The hydrogenation of safrole to dihydrosafrole (DHS)
- -The reaction of DHS with formaldehyde and hydrochloric acid to furnish chloromethyl DHS.,
- -The reaction of chloromethyl DHS with butyl carbitol.

B. Registered Products, Uses, and Tolerances

Piperonyl butoxide is employed as a pesticide synergist in about 4,200 federally registered formulated products manufactured by approximately 900 registrants. In the United States, the principal manufacturers of technical PB are Fairfield American (formerly FMC), Alpha Laboratories, Inc., Prentiss Drug and Chemical Company, Inc., and McLaughlin, Gormley, King Company. The Agency has developed a yearly aggregated produiduction estimate of 600 to 1,200 thousand pounds active ingredient (EPA, 1980).

Tolerances for residues of PB have been established on raw agricultural commodities as follows (40 CFR 180.127):

20 ppm from post harvest application in or on barley, birdseed mixtures, buckwheat, corn (including popcorn), rice, rye and wheat,

8 ppm from post harvest application in or on certain fruit, nut, seed and grain crops (see 40 CFR 180.127 a complete crop list),

3 ppm in meat, fat, and meat byproducts of poultry,

1 ppm in eggs,

0.25 ppm from postharvest application in or on potatoes,

0.25 ppm (reflecting negligible residues in milk) in milk fat,

0.1 ppm (negligible residue) in the meat, fat and meat byproducts of cattle, goats, hogs, horses and sheep,

An exemption from the requirement for a tolerance has been granted PB when application is made "...to growing crops in accordance with good agricultural practice." [40 CFR 180.1001(b)(4)] The apparent inconsistency created by permitting an exemption from the requirement for a tolerance for field application, but establishing tolerances for post harvest applications will be addressed at time of product re-registration.

PB may additionally be applied in food processing and food storage areas "provided, that the food is removed or covered prior to such use" (21 CFR 193.370(a)(5).

C. Exposure

Although in recent years there has been a trend toward the use of unsynergised synthetic pyrethroid formulations, PB, in combination with pyrethrum, remains common in the marketplace. PB is formulated into total release pressurized products, intermittent aerosols, fogging concentrates, emulsions, dusts, wettable powders and other more specialized products. In considering potential human exposure, the Agency believes that direct inhalation is the most significant route. There is, additionally, some potential for dietary exposure through the use of aerosol products in food handling and processing areas of commercial establishments, home garden use and post harvest treatment of certain fruits, vegetables and grain.

The Agency has not located any test data relative to either inhalation or dietary exposure. Theoretical inhalation values, however, have been calculated (Brown, N.C., 1970). The specific formulation types and application rates utilized in developing the theoretical values are consistent with certain products still in use within the United States. Although not fully adequate for the development of an exposure analysis, the Agency will rely upon these data until such time as additional data might be necessitated by the acquisition of positive chronic toxicity data.

III. Piperonyl Butoxide as a Potential RPAR Candidate

A. Introduction

As previously noted, piperonyl butoxide was suspected by the Agency of meeting the risk criteria established under 40 CFR 162.11. Although the potential oncogenicity of PB initiated Agency review, additional concerns evolved in response to the Agency's comprehensive review of available toxicological data. The added areas of concern were co-oncogenicity (co-carcinogenicity), mutagenicity, reproductive effects, teratogenicity, and metabolism. The individual areas of concern and the relevant data are discussed below.

B. Oncogenicity

40 CFR section 162.11 (a)(3)(ii)(A) provides that a "rebuttable presumption shall arise if a pesticide's ingredient(s)...(i) induces oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation, or dermal exposure..." Section 162.3 (bb) defines the term oncogenic as "the property of a substance or a mixture of substances to produce or induce benign or malignant tumor formation in living animals."

1. Sources Originally Suggesting PB as an Oncogen

Innes et al. (1969) administered PB or Butacide® (the trade name for PB) to B6C3F1 and B6AKF1 hybrid mice by both oral and subcutaneous routes. PB was administered orally by gavage to one group at 100 mg/kg in 0.5 percent gelatin for three weeks, then switched to 300 ppm dietary for up eighteen months. PB was administered subcutaneously to a second group at 100 mg/kg. Butacide® was administered to the orally dosed group at a rate of 464 mg/kg by gavage for three weeks. From week three to the termination of the study at eighteen months, Butacide® was administered at 1,112 mg/kg dietary in 0.5 percent gelatin. A second Butacide® group received subcutaneous injections of 100 mg/kg in corn oil. The animals were examined for incidences of hepatomas, lymphomas, and pulmonary tumors. The investigators observed an "elevated incidence in an uncertain range" in the treated group when compared to controls and classified PB as a compound requiring additional study as a "tumorigen".

Innes et al. based their conclusion on the results reported in a more detailed report by Bionetics Research Labs, Inc. (1968). That report clarifies the term "uncertain range" used by Innes et al. There was an increased incidence of total tumors in male B6C3F1 mice treated subcutaneously with Butacide®. There was also an increased incidence of reticulum cell sarcoma in the same sex and strain of mice treated orally with PB. This was regarded as evidence by Innes et al. that an additional study was needed. Since the increased

incidence of reticulum cell sarcomas was not seen in mice given PB subcutaneously, the classification of PB as an active "tumorigen" was uncertain. In the case of Butacide®, the increased incidence of total tumors was statistically significant when the treated group was compared to pooled controls, but not when compared to the matched controls. Other chemicals tested by Innes et al. were found to increase the incidence of tumors above controls by both oral and subcutaneous routes of administration. These chemicals were classified as active or "tumorigenic." Thus, Innes et al. recommended that PB be investigated further for potential carcinogenicity.

Agency scientists (Gardner, R., 1979a and 1980) reviewed Innes' study and found it to be scientifically valid. However, in the case of Butacide®, no statistical significance is found when the treated group is compared with the appropriate matched untreated control group or the solvent (corn oil) control group. The increased incidence of total tumors is only statistically significant when the treated group is compared to pooled controls ($p=0.05$). Thus, the increased incidence of total tumors cannot be clearly associated with Butacide® administration.

The Agency's Carcinogen Assessment Group (Byrd, D.M., 1981) stated that the Innes study showed that PB caused reticulum cell sarcoma in one strain of treated male mice through one route of administration. The Agency agreed with the authors that further study was necessary to more completely evaluate PB's potential carcinogenicity.

In his review of studies on the toxicology of pesticides, Mrak (1969) discussed the findings of Innes et al. and supported their conclusion that further evaluation of PB as an oncogen was indicated. He recommended that the possible interaction of PB with a variety of toxic substances be investigated and that tests on mixed function oxidase inhibition be included in the safety evaluation.

2. Other Oncogenic Studies

This section summarizes additional oncogenic studies which the Agency reviewed.

a. The NCI Study

The National Cancer Institute (NCI) studied PB in 1978 in its Carcinogenesis Testing Program. Technical grade PB was tested.

Two groups of 50 Fisher 344 Rats of each sex were given PB in the diet at either 5,000 or 10,000 ppm for 107 weeks. Matched controls consisted of 20 untreated rats of each sex. One hundred B6C3F1 mice of each sex were administered PB at either 2,500 or 5,000 ppm for 30 weeks. Then, because some mice died

the doses were reduced to 500 and 2,000 ppm, respectively, for 82 more weeks. Matched controls consisted of 20 untreated mice of each sex. Survival was 80 percent of the original; thus sufficient numbers were at risk for the possible development of late-appearing tumors. All the rats and mice were sacrificed at the end of the period of administration of the test chemical and were examined.

NCI (1978) found that in the female rats there was a statistically significant incidence ($p=0.020$) of lymphomas in the high-dose group (15 of 50) when compared to the controls (1 of 20). The incidence of lymphomas in historical-control rats in other studies at the same laboratory, however, has been 19/191 (10%), 7/20 (35%), and 6/20 (30%). Thus, NCI concluded that the incidence of lymphomas in the control female rats in this particular assay may have been abnormally low. The occurrence of a higher incidence in the dosed groups, therefore, could not be related to the administration of FB (Byrd, D.M., 1981). Although the NCI did not perform a statistical test of significance to compare the incidence of lymphomas of the treated female rats with that of the historical controls, the Agency's Cancer Assessment Group (CAG) did perform such a test and found $p = 7 \times 10^{-3}$ (Haberman, B.H., 1981) (significant at 0.001 level).

In the male rats, a significant decrease with increasing dose of FB was observed in the incidence of neoplastic nodules of the liver and of adenomas or carcinomas of the pituitary. The incidence of these tumors in the control group exceeded that of the dosed groups.

In the female mice, a significant decrease with increasing dose of FB was observed for the incidence of lymphomas. The incidence of this tumor in the control group exceeded that of the dosed groups.

In the male mice, adenomas of the eye or lacrimal gland occurred at incidences that were dose-related, but in direct comparisons, the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 0/49, high dose 4/50). NCI would expect, under normal circumstances, the spontaneous appearance of a tumor in 1 to 3 out of 20 controls; therefore, an incidence of 4 out of 50 in the high dose group could have occurred merely by chance (Ward, 1980). The NCI report stated that "the occurrence of this tumor in the male mice was not clearly related to administration of the test chemical."

NCI's general assessment of its testing program states that negative results do not necessarily mean the test chemical is not carcinogenic, since the experiments are conducted only under a limited set of circumstances. Positive results would demonstrate that the test chemical is carcinogenic for animals under the test conditions and would indicate that exposure to the chemical is a potential risk to man.

NCI concluded that "under the conditions of this bioassay, piperonyl butoxide was not carcinogenic for Fisher 344 rats or B6C3F1 mice."

Agency scientists (Roger, G., 1979b and 1980), including those in the CAG (Byrd, D.M., 1981), reviewed and evaluated the NCI Bioassay and found, however, a statistically significant increase in the occurrence of lymphomas in treated female rats when compared to matched controls, or historical controls from other NCI studies. The male control rats had an unusually high incidence (45%) of lymphomas. Goodman (1979), for example found a 12 percent incidence of lymphomas for pooled male control rats. (The NCI study does not describe the incidence in pooled male control rats.) The high variability in lymphoma incidence in male and female control rats in the NCI study makes it difficult to interpret the results of this carcinogenicity bioassay. The utility of the study must, further, be viewed from the perspective that the potentially useful findings relate only to one sex and one species.

Agency scientists (Gardner, R., 1979b and 1980; Byrd, D.M., 1981) also noted an increased occurrence of adenomas of the lacrimal gland in treated male mice when compared to matched controls. However, the number of tumors was small and the findings could have occurred by chance variation, as Dr. Ward (1980) of NCI stated.

Agency scientists concluded that further testing is necessary; the results of the NCI Bioassay are not definitive enough to judge whether PB is positive or negative for carcinogenicity. The Agency, at the time of development of protocols for the required testing, will assist in identifying rat and mouse strains having historically a low incidence of spontaneous lymphomas. This, coupled with increased test group sizes, is anticipated to provided more definitive results.

b. The Hunter Long-Term Feeding Study

Hunter et al. (1976) conducted a long-term feeding study with PB and pyrethrum, the insecticide most often used with PB. Pyrethrum and PB mixed in a ratio of 1 to 5 were administered to 45 male and 45 female Sprague-Dawley rats. Untreated

controls also consisted of 45 Sprague-Dawley rats of each sex. After two years of treatment, examination of tissues for tumors revealed histopathological changes in the treated as well as the control groups.

Observation revealed, in both treated and control groups, enlarged pituitaries (some with tumors), subcutaneous masses, areas of fibrosis, liver changes, testicular atrophy, ovarian cysts, and tumors of the mammary gland. There was no significant difference in the frequency of tumors in the treated group as compared to the controls.

Agency scientists Edwards, W.T. (1978) reviewed this study and found it to be valid; however, because animals were fed a mixture and not PB alone, no conclusions can be made about the carcinogenicity of PB itself. Again, the Agency concluded that further testing is necessary to determine PB's potential for causing carcinogenic effects.

3. Possible Oncogenic Contaminants or Impurities

The Agency's Guidelines provide that "The composition of each lot of the test substance shall be determined, including the name and quantities of known contaminants and impurities, as far as is technically feasible. The determination shall include quantities of unknown materials, if any, so that 100 percent of the test sample is accounted for." (43 FR 37352)

Brown, (1970) indicated that the first step in the manufacture of PB is the hydrogenation of safrole to dihydrosafrole, both of which are known carcinogens (Innes et al. 1969). Theoretically, safrole or dihydrosafrole could possibly contaminate the final PB technical product. Since the Agency has no information on the details of the manufacturing process for PB, the registrant(s) must supply this information on product chemistry in order to rule out the possibility that technical PB is contaminated with known carcinogens. This information is necessary so that the Agency can take the appropriate steps to protect the public in the event that there is product contamination. In addition, when testing PB for oncogenic effects, it is imperative that the most pure technical be used. In this way, any resulting toxic effects can be attributed to the test chemical alone and not to any product contamination.

C. Cocarcinogenicity (Co-oncogenicity)

One study investigated the possibility that PB was a co-carcinogen.

Co-carcinogenicity is a property of two chemicals, not necessarily carcinogenic by themselves, that induces tumors when the chemicals are administered together. Epstein et al. (1979a) hypothesized that PB mixed

with "Freons" (fluorocarbons used as propellants with pressurized aerosols of pesticides), might induce carcinogenicity; that is, PB could be a cocarcinogen. In order to study this hypothesis, neonatal Swiss mice were subcutaneously injected in the nape of the neck with a 5 percent solution of PB in redistilled tricaprylin (the solvent) in volumes of 0.1 ml at ages one and seven days and 0.2 mls at ages 14 and 21 days. "Freons" 112 and 113 were injected in combination with PB to these animals and also separately to two other groups. Control animals were injected with the solvent alone. Animals were allowed to survive until the experiments ended in 50 to 52 weeks. It was found that hepatomas occurred only in males, the highest incidence being in the groups given both PB and "Freons." The results are summarized in Table I.

The hepatoma incidence in male mice for the combination treatment was 17 percent (Freon 113 & PB) and 31 percent (Freon 112 & PB) in contrast to a 5 percent (Freon 113) and 0 percent (Freon 112) incidence in the separate treatment groups. The incidence of obstructive uropathy in the solvent controls was equal to or higher than the incidence in groups given Freons 112 and 113 alone. Epstein et al. suggested that PB may alter the metabolism of "Freon," perhaps by inhibiting dechlorination. The authors did not evaluate the statistical significance of increases in tumor incidence for animals treated with PB alone or in combination with the two Freons.

Agency scientists (Mishra, L.C., 1978 and Gardner, R., 1980) reviewed this study and applied statistical tests for significance of the data. The results are found in Table II. There was an apparently statistically significant increased incidence of hepatomas in neonatal Swiss mice treated with PB and Freon 112 when compared to that of the solvent control group ($p=0.047$). These results do not clearly associate the increased incidence of hepatomas with PB and Freon 112 because there are no negative control data with which to draw a comparison against the solvent control group. The incidence of tumors in the male solvent controls was 8 percent (4/48). This incidence was significantly different from that of the female solvent controls (0/68). Also, some animals, which were severely autolysed and cannibalized were not examined histopathologically. Had they survived, the significance of the tumor incidences may have been different.

The incidence of tumors in animals treated with PB and Freon 113 was not significantly different from the solvent control group, though the incidence of hepatomas in the animals treated with PB and Freon 112 was, as stated above, significantly different from the solvent controls.

This suggests the possibility of specificity with regard to the Freons with which PB could be co-carcinogenic. However, when the results of the two PB combinations are compared, no significant difference is found ($p=0.222$). The number of tumors in the PB plus Freon 112 group was significantly different from the number in the PB plus Freon 113 group.

Agency scientists (Mishra, L.C., 1978 and Gardner, R., 1980) indicated that the results of this study do not clearly establish whether PB is or is not a co-carcinogen with the freons tested. The Freons 112 and 113 used in this experiment are not the freons used as propellants with aerosol cans of pesticides containing PB. Those freons are Freons 11 and 12. The Agency is not aware of any studies which tested PB and Freons 11 and 12 for co-carcinogenicity.

At the present time, the Agency does not have a policy which addresses the problem of potential toxicity due to interactions of chemicals. There is no policy indicating how co-carcinogenicity might be assessed and co-carcinogenicity is not cited in the guidelines as a risk criterion which would initiate the RPAR process. Also, current risk assessment methods are inadequate for estimation of human risk based on this type of laboratory data. Therefore, the Agency will not at this time require the registrants to perform further tests to assess PB's potential for causing co-carcinogenic effects.

D. Mutagenicity

40 CFR 162.11 (a)(3)(ii)(A) provides that a rebuttable presumption shall arise if the pesticide's ingredient(s), metabolite(s), or degradation product(s) induce "mutagenic effects, as determined by multitest evidence..." The Agency reviewed four papers discussing PB and mutagenicity.

Epstein et al. (1972) used the dominant lethal test in order to assess the possible mutagenicity of PB. Male Swiss mice (Charles River Breeding Laboratories) were injected once intraperitoneally with either 200 mg/kg (7 animals) or 1000 mg/kg (9 animals) PB, or were given 1000 mg/kg orally by gavage over a period of 5 days (10 animals). Following treatment, each mouse (including solvent controls) was caged with untreated virgin females which were sacrificed 13 days after presumptive mating.

The females were scored for numbers of pregnancies, numbers of fetal deaths, and numbers of live implants. These scores were compared with the controls. PB produced significant changes in the numbers of pregnancies resulting from mating with only those males injected at a lower subcutaneous dose. PB also produced a significant number of early fetal deaths from pregnancies resulting from males given PB by both routes of administration, but not at the higher injected dose. Total live implants were within control limits for both routes of administration.

The authors discuss the use of increased early fetal deaths as an unequivocal measure of dominant lethal mutations (mutagenicity). However, according to the authors, PB cannot be classified a mutagen without further verification. They state that the effect was equivocal due to "internal inconsistencies." In the case of males treated

intraperitoneally, an effect on early fetal deaths was demonstrated only at the lower dose. The authors assigned a "borderline significance at the 5% level" to the effect of higher early fetal death in animals administered PB.

Agency scientists (Mauer, L., 1978b and 1980) evaluated the study and stated that because of the small number of animals used, administration of only one dose orally, dose reversal in the number of early deaths following intraperitoneal injection, and the borderline significance of 5 percent for an oral dose, the Agency would require additional testing before PB could be evaluated for mutagenic potential.

Ashwood-Smith et al. (1972) tested the mutagenicity of PB in bacteria. A 10 percent concentration of Butacide® (containing 80 percent PB and 20 percent "related compounds") was added to cultures of the auxotrophic mutant WP2 Try-(tryptophan requiring) of Escherichia coli. Mutants/revertants not requiring tryptophan were scored after 48 hours of incubation. PB was reported to be negative in this test for mutagenicity. Agency scientists (Mauer, L., 1978a and 1980) commented that the test was performed without a mammalian metabolic activation system, which might generate mutagenic metabolites. Also, the high concentration (20 percent) of unidentified compounds could have interfered with the potential for direct mutagenicity at the single concentration used. Therefore, the results of the study are inconclusive and further testing is necessary.

Friedman and Sanders (1976b) used an in vivo mouse assay to determine what effects PB might have on the metabolism and mutagenicity of dimethyl nitrosamine (DMN) (which requires metabolic activation for mutagenic activity). PB was injected intramuscularly (i.m.) into male Swiss-Webster ICR mice at doses of 10, 40, or 640 mg/kg. DMN, at a dose of 500 mg/kg, was injected i.m. at the same time as an intraperitoneal injection (45 minutes later) of Salmonella typhimurium G-46 cells. It was found that PB inhibited liver demethylase activity resulting in a decrease in metabolism of DMN and a consequent reduction of DMN mutagenicity, as shown by a decrease in the number of Salmonella revertents compared with the number when DMN was administered alone. Agency scientists (Mauer, L., 1978d and 1980) in their review stated that since PB was not administered alone, the authors could not make any conclusions about the mutagenicity of PB itself.

Friedman and Staub (1976) proposed the inhibition of mouse testicular DNA synthesis by mutagens as a potential mammalian assay for mutagenesis. In their study, five male Swiss mice were given 640 mg/kg PB by intraperitoneal injection. Three hours later each mouse received 10 microcuries of radioactively labeled thymidine. The mice were then sacrificed and the testes were examined for DNA concentration and radioactivity. There was no difference found between the treated group and controls.

In their review of the paper, Agency scientists (Mauer, I., 1978c and 1980) pointed out that there may not necessarily be a correlation between mutagenic activity and inhibition of testicular DNA synthesis. In addition, the Agency submits that the expected exposure may not cause assimilation into the testes. This type of test may, therefore, have little or no relevance for assessing mutagenesis.

The results of these four studies are inadequate to assess whether the criterion for mutagenicity has been met or exceeded; further testing is necessary to assess potential mutagenic effects of PB.

E. Chronic Toxicity

40 CFR 162.11 (a)(3)(ii)(B) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s)" produces any other chronic or delayed effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety."

1. Reproductive and Fetotoxic Effects

Because products containing PB have numerous home and garden uses, it may be anticipated that men and women of reprocutive age will be exposed to a significant degree. It is due to this exposure potential that reproductive, fetotoxic and teratogenic effects take on added significance.

A single reproduction study, Sarles and Vandegrift (1952), has been located and reviewed by Agency scientists. The study has been found deficient. The test group sizes were small at the outset of the study, with subsequent sacrifices and high mortality further diminishing the population. The two highest dose levels (10,000 and 25,000 ppm) decreased food consumption and compromised nutrition so that compound related effects could not be evaluated. In addition, only two litters were used for the second and third generations.

The above noted study is not sufficient as an indicator as to whether or not the criterion for reproductive effects has been met or exceeded. The Agency, therefore, has determined that an additional study, to be conducted by the registrant, is required (Gardner, R., 1981b.)

2. Teratogenic Effects

Two studies dealing with PB's potential teratogenic effects have been located by the Agency.

In a study conducted by IBT (Industrial Bio-Test) (Adler, G. and S. Smith, 1978) for McLaughlin Gormley King Company (1976), albino rats were used to assess the effects of PB on mortality, behavior, reproduction and teratogenicity. Agency scientists could not comment on this study, because results of studies done by IBT are currently suspect due to questionable laboratory practices. All IBT studies must be audited before they can be validated by the Agency. This particular study is currently being audited and will then be reviewed by the Agency. The Agency does not, however, consider this study to be critical. The following study by Khera et al. (1979) provides information sufficient to satisfy current Agency needs.

Khera et al. (1979) investigated PB's teratogenic potential utilizing female Wistar rats mated with proven males. Each dose group consisted of 18-20 mated females. Technical grade PB in distilled water was administered once daily by esophageal intubation from days 6-15 of gestation. The doses were 62.5, 125, 250, and 500 mg/kg. The females were killed on day 22, and the carcasses were weighed after the uterine contents were removed. Fetuses were weighed and examined for viability and external malformations. Resorptions and dead fetuses were recorded. Two-thirds of the fetuses were stained with alizarin red and examined for skeletal defects. The remaining one-third were fixed and freehand sectioned and examined for visceral anomalies.

Doses of 500 mg/kg of PB produced no signs of toxicity or statistically significantly reductions in body weight gain in dams during gestation.

PB, at the maximum dose tested (500 mg/kg), manifested no prenatal effects. The incidence of anomalies in treated groups was comparable to that in the controls.

The Agency has concluded that the Khera et al. study serves as an adequate indication of PB's nonteratogenicity (Gardner, R., 1981). Although Agency guidelines customarily require teratogenicity testing in at least two mammalian species (43 FR 37383), the negative findings of the Khera et al. study do not trigger the need for additional data in relation to the Agency's investigation of PB as a potential RPAR candidate. Additional teratogenicity testing may, however, be required in relation to future reregistration actions.

3. Metabolic Effects: Mixed Function Oxidase Inhibition

Several studies dealt with the inhibitory effect of PB on mixed function oxidases (mfo) in microsomes in various tissues of different animals. Microsomal enzymes, in metabolizing a given chemical other than PB, cause the chemical to be activated or inactivated. If a

metabolite of the chemical is the active form, PB could diminish the chemical's effect by inhibiting breakdown of the chemical. If, on the other hand, the chemical itself is the active form, then PB could enhance the chemical's effect, by inhibiting its breakdown.

a. Inhibition Studies *in vitro*

Generally, in the *in vitro* inhibition studies, microsomes containing the mfo's were prepared from the livers of rats, mice, or hamsters and were incubated with PB and various test enzymes and chemicals.

Graham et al. (1970), using the livers of rats, found that PB at 6×10^{-5} M *in vitro* interfered with the action of the enzymes aminopyrine demethylase and aniline hydroxylase by competitive inhibition.

Friedman and Couch (1974) confirmed these findings using mice which were administered 500 mg/kg PB by intraperitoneal injection (i.p.).

Friedman and Epstein (197?) observed that PB administered i.p. at 2.5 mg/kg or 10 mg/kg first caused an induction of microsomal aminopyrine demethylase activity and later an inhibition, 48 hours after treatment with PB. Hodgson and Casida (1961), in their review article on microsomal metabolism found that PB also inhibited the metabolism of some N,N-dialkyl carbamates in rat liver. Using hamsters, Hinson et al. (1975) found that PB administered to rats at 1500 mg/kg i.p. inhibited the action of the enzyme p-chloroactanilide (PCAA) hydroxylase, causing a decrease in the production of N-hydroxy-PCAA, a metabolite of PCAA.

Baker (1974a) in his abstract on the mfo system determined that PB (dose not specified) inhibits microsomal enzyme activity by acting both as an alternate substrate (competetive inhibition) and by binding to Cytochrome P-450, a component of the microsomal mfo system. The review by Hodgson et al. (1973) supports Baker's conclusion.

These *in vitro* studies show that PB inhibits microsomal mfo's. Agency scientists (Marquardt, G.M., 1978d,e,h,i,j,k,l) reviewed these studies and commented that the results and conclusions are valid.

b. Inhibition Studies - *in vivo*

In vivo studies of mfo inhibiton by PB confirm the *in vitro* findings. Generally, PB was orally or intraperitoneally

administered to rats or mice before treatment with test chemicals, such as hexobarbital, zoxazolamine, and dietary hydrocarbons.

Fine and Molloy (1964) found that PB administered to mice at 50 mg/kg i.p. caused a three-fold increase in sleeping times due to prolonged action of hexobarbitol. Fujii et al. (1968) confirmed this finding and also reported an increase in paralysis times, due to prolonged action of zoxazolamine. Alvro and Fishbein (1970) found an increase in the concentration of n-octadecane and n-nonacosane, dietary hydrocarbons, in the blood and tissues of rats pre-treated p.o. with 1 g/kg PB.

Conney et al. (1972) administered PB to rats i.p. at 2000 mg/kg, 500 mg/kg, and 50 mg/kg. It was found that 50 mg/kg, had no effect on antipyrine metabolism. At the higher doses, antipyrine metabolism was inhibited. In mice, the no-observed-effect-level (NOEL) for inhibition of antipyrine metabolism was 0.5 mg/kg PB; thus, mice are 100-fold more sensitive than rats to the effects of PB.

Results of all in vivo studies reviewed indicate PB inhibits the mixed function oxidase system of metabolizing enzymes, which would normally break down and inactivate the test chemicals. The activity of the test chemicals is, therefore, increased. Agency scientists (Marquardt, G.M., 1978a,g,m,o) reviewed these studies and found their results and conclusions to be valid.

c. Inhibition Studies with Known Carcinogens or Mutagens

Falk et al. (1965) investigated the influence of PB in rats on the metabolism of benzo(a)pyrene, a known carcinogen. Since PB inhibited the hepatic metabolizing enzymes, there was a decrease in the elimination of benzo(a)pyrene in the bile.

In a similar experiment by Conney et al. (1972) 2000 mg/kg PB administered i.p. to rats caused a decrease in the metabolism of benzo(a)pyrene. No effect was found when using 50 mg/kg PB.

In the study by Friedman and Sanders (1976b), already discussed in the mutagenicity section of this document, PB was found to diminish the mutagenic effects of dimethyl-nitrosamine.

No studies were found indicating that PB enhanced any mutagenic effects.

d. Inhibition Studies in Humans

Although PB causes inhibition of mfo's in laboratory animals, it is important to know if the same result could occur in humans exposed to PB.

Conney et al. (1972) administered 0.71 mg/kg PB orally to human volunteers and found that PB did not inhibit the metabolism of the chemical antipyrine. They stated that "...since this dose of PB is 50 times greater than the daily exposure received by individuals who use sprays extensively in an enclosed area, the environmental exposure of people to PB is probably insufficient to inhibit the function of the microsomal enzymes." Conney et al. did not indicate their source of daily human exposure data, but their comparisons are consistent with those data published in the previously mentioned review article by Brown (1970) (see page 4).

Agency scientists (Marquardt, G.M., 1978m) commented that the protocol was scientifically acceptable. Assuming that the exposure data referred to were valid, the authors' conclusion that the environmental exposure to PB was probably insufficient to inhibit the function of the microsomal enzymes is valid under the conditions of that experiment. However, since this is only one study, using one dose level to assay the metabolism of a single chemical (antipyrine), Agency scientists (Brantner, J., 1979) could not generalize from the authors' conclusions.

None of the studies describing the metabolic effect of PB on mixed function oxidase inhibition met or exceeded the risk criteria outlined in 40 CFR 162.11. The concentrations of PB used in the experiments were extremely high; it would be unlikely that humans would be exposed to such high levels (Brown, 1970). Since mfo inhibition by PB has been so thoroughly studied, no further testing of this metabolic effect is necessary.

4. Other Chronic Effects

a. Biological Fate of PB and its Metabolites

In order to trace the biological fate of PB, Fishbein et al. (1969) treated male Sprague-Dawley rats intravenously with radioactive PB (dosages not reported). After eight hours, samples of lungs, liver, kidney, heart, fat, blood, and other tissues were examined for radioactivity. Although there was a wide distribution, approximately 12-17 percent of the

administered dose was found in the lungs. Agency scientists reviewed this study and made the following statements concerning the metabolism of PB:

- -Metabolites of PB are rapidly excreted into the bile of treated rats.
- -The rates of urinary excretion of PB metabolites were less than the rates for appearance in the bile.
- -Biliary and urinary metabolites of PB were only partially characterized.
- -Approximately 40 percent of the administered dose of PB appeared as CO_2 in the air expired by the rats.
- -The lungs contained relatively large amounts of PB (12-17 percent) present primarily in the unchanged form.

The Agency (Marquardt, G.M., 1978c) has determined that more study is needed to substantiate these findings and to suggest possible modes of action by which PB might "preferentially" accumulate in the lungs. Additionally, as chronic inhalation testing is extraordinarily difficult, the Agency has elected to request only chronic feeding studies. Metabolism data, enabling the Agency to draw conclusions from only oral exposure studies, must be developed.

b. Pathology

In the two year feeding study by Hunter et al. (1976), already discussed in the oncogenicity section of this document, the rats, which had been treated with pyrethrum and PB administered together, were also studied for any clinical abnormalities.

The males had been given on the average 15.9 mg/kg/day PB. The doses of insecticide-to-synergist were in a ratio of 1:5, analogous to some formulated products. Urinalyses indicated a higher percent of treated females had protein in their urine than controls did ($p<0.01$). A lower lymphocyte count was found in treated males, although all groups had values within the normal range.

Agency scientists (Edwards,W.T. 1978) indicated that although positive results may have been age-related rather than treatment-related, it is possible that the clinical effects may have resulted from the combination of pyrethrum and PB. They commented further interpretation is limited because only one dose level was used. At least three dose levels and a control are needed, with the highest dose clearly showing an effect.

c. Mortality

Friedman and Sanders (1976a) studied the effect of PB on mortality in Swiss albino mice due to the known carcinogen dimethylnitrosamine (DMN). The mice were injected intraperitoneally with 640 mg/kg PB 45 minutes before injection with 29.6 mg/kg DMN. LD_{50} values were determined on the seventh day after treatment. PB treatment did not significantly alter the mortality of the DMN-treated mice as compared to controls given DMN alone; there was similar mortality in both groups.

Agency review (Marquardt, G.M., 1978f) of these data indicate that the acute mortality produced by DMN is probably mediated by a different mechanism of action than that causing oncogenic or mutagenic effects. Though PB has been shown to inhibit DMN demethylase (the enzyme catalyzing the formation of the active DMN metabolite), the DMN- produced lethality is probably not mediated by this mechanism.

Epstein et al. (1967b) studied the toxicity (mortality) of Freon 112, Freon 113, griseofulvin (an antifungal antibiotic), and benzo[a]pyrene with and without PB. Random-bred Swiss infant mice (ICR/Ha) were injected subcutaneously with Freon 112 or Freon 113 (0.1 - 0.2 ml/animal), griseofulvin (0.125-1.0 mg/animal) or benzo[a]pyrene (10 ug/animal) with or without PB (0.1 mg/animal at an initial dose of 2500 mg/kg). The percent mortality was recorded on days 1, 7, 14, and 21. It was found that mortality due to the test chemicals was markedly enhanced by treatment in combination with PB. Agency scientists (Marquardt, G.M., 1978b) concluded that this effect was presumably due to the PB- inhibition of the microsomal enzymes metabolizing these compounds, thus increasing their toxicity.

There is the theoretical possibility (Mrak, S., 1979) that PB could enhance the toxic effects of some drugs or chemicals in the environment and cause adverse effects in humans, but it would be impossible to test PB in combination with every one of these chemicals.

F. Effects on Fish and Wildlife

40 CFR Section 162.11(a)(3)(ii)(c) establishes a criterion to protect against significant population reductions in local, regional, or national populations of nontarget organisms or fatality to members of endangered species.

The Agency has determined there is low risk to fish and wildlife based primarily upon the patterns of use and minimal exposure potential (Bushong, C. 1979).

IV. Conclusions and Recommendations

A. Summary of Conclusions

With respect to piperonyl butoxide as an RPAR candidate, the Agency concludes that the presently available data on oncogenicity, mutagenicity, and chronic toxicity including reproductive and teratogenic effects, do not support a "Rebuttable Presumption Against Registration" of pesticide products containing piperonyl butoxide.

1. Oncogenicity

Results of the studies by Innes et al. (1969) in mice and the NCI Bioassay (1978) in mice and rats are not adequate to determine whether PB by itself is associated with the production of tumors. Further testing by the registrants will be necessary to properly assess the potential oncogenic effects of PB.

- Testing must be performed as described in 43 FR 37379 with the strain of rat and mouse to be determined in consultation with the Agency.

- In accordance with 43 FR 37352 information on any contaminants and impurities must be submitted.

- Monitoring for oncogenic effects can occur while also assessing other chronic effects as explained under item 7 of this section.

2. Product Chemistry

Because safrole and dihydrosafrole, two known carcinogens, are involved in the manufacture of technical PB, the registrant must supply the Agency with information on the manufacturing process and the contaminants or impurities found, as far as is technically feasible, in the final PB product.

- Information on the product and its manufacturing process must be submitted by the registrants in accordance with 43 FR 29709.

3. Mutagenicity

The results of the four studies by Epstein et al. (1972), Ashwood-Smith et al. (1972), Friedman and Sanders (1976a), and Friedman and Staub 1976) are inconclusive. Further testing must be done by the registrant to properly assess PB's potential mutagenic effects.

- For data on gene mutation, the registrants must conduct an Ames assay using 5 strains of bacteria and either a point mutation in mammalian cells or a Drosophila test for sex-linked recessive lethals in accordance with 43 FR 37389.

- For chromosome aberration, the registrants must conduct an in vivo cytogenetics test in mice or rats, a dominant lethal test in accordance with 43 FR 37389, and either an in vitro cytogenetics test in human cells or a cytogenetic analysis of blood cells from exposed persons as provided in 43 FR 37401, Addendum 3, footnote 2, which states "...EPA may require data in addition to those specified in the proposed guidelines to assess the risks to humans."

- For DNA damage/repair, the registrant must conduct a sister chromatid exchange in mammalian cells and either a yeast test for mitotic recombination or a bacterial DNA repair assay in accordance with 43 FR 37392.

- In addition, the registrant must conduct a micronucleus test in mice for possible non-disjunction and a mammalian cell transformation assay as provided in 43 CFR 37399, Addendumn 3, Assessment of Human Risk, No. 8, which states, "A chemical may cause mutagenic effects by mechanisms such as disturbed segregation of chromosomes and suppression of DNA repair mechanisms. Considerations other than those described above will apply to the evaluation of risk from mutations caused by such mechanisms."

4. Reproductive Effects Testing

   - As piperonyl butoxide possesses a high exposure potential in relation to humans of reproductive age, and inadequate data are available, the Agency has determined that the registrant(s) must conduct a three-generation reproduction study in accord with 43 FR 37384.

5. Teratogenic Testing

   - As a result of the review of Khera et al. (1979), the Agency finds no indication that PB possesses any teratogenic potential in rats. Although Agency guideline requirements have not been fulfilled with regard to testing in two species, 43 FR 37382, no additional data will be required at this time.

6. Metabolite Testing

   - The Agency believes that the single greatest potential for exposure to PB rests with the inhalation of spray mist from aerosol and pressurized spray formulations. As chronic inhalation testing is extraordinarily difficult, the Agency has elected to request only chronic feeding studies. Metabolism data, enabling the Agency to draw conclusions from only oral exposure studies, must, therefore, be developed. The registrant(s) must submit studies to assess the uptake and metabolism of PB as described in 43 FR 37394.

7. Chronic Feeding Study

- Because Hunter et al. (1976) suggested that administration of PB plus pyrethrum may have produced clinical signs of toxicity, it is necessary for the registrants to conduct a chronic feeding study using PB alone as described in 43 FR 37375. As specified, monitoring for effects due to repeated exposure to a pesticide can be combined with an oncogenic evaluation. Thus, as long as standards for both types of testing are met, the registrant(s) may monitor both types of effects during one chronic feeding study.

A Notice will be sent to the registrants pursuant to FIFRA section 3(c)(2)(B) informing them of the requirement for performing these tests and describing in more detail the protocols they must follow and the actions which they must take to comply with the Notice.

Table I: Cocarcinogenicity of PB and Freons (Epstein et al., 1967a)

Treatment group	Number with hepatomas of total examined	
	Males	Females
Solvent control	4/48	0/68
Freon 112	0/17	0/19
Freon 113	1/21	0/20
Piperonyl butoxide (PB)	0/20	0/36
Freon 112+PB	4/13	0/24
Freon 113+PB	3/18	0/24

Table II: Statistical Significance of Epstein Study

Comparison	p value
(Freon 112 + PB) with solvent control	0.047 a/
(Freon 113 + PB) with solvent control	0.255
(Freon 113 + PB) with (Freon 112 + PB)	0.222
(Freon 113 + PB) with Freon 113	0.208

a/ Significant difference (P less than 0.05)

VII.

Piperonyl Butoxide

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